AWARD NUMBER: W81XWH-14-1-0558

TITLE: Human iPSC-Derived GABA-Ergic Precursor Cell Therapy for Chronic Epilepsy

PRINCIPAL INVESTIGATOR: Ashok K. Shetty, Ph.D.

CONTRACTING ORGANIZATION: Texas A&M University System
College Station, TX 77845

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sep 2014 to 29 Sep 2015

4. TITLE AND SUBTITLE

Human iPSC-Derived GABA-Ergic Precursor Cell Therapy for Chronic Epilepsy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0558

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Ashok K. Shetty, Ph.D.

E-Mail: shetty@medicine.tamhsc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

THE TEXAS A&M UNIVERSITY
SYSTEM
200 TECHNOLOGY WAY, STE 2079
COLLEGE STATION TX 77845-3424

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to examine whether grafting of medial ganglionic eminence (MGE)-like precursors generated from human induced pluripotent stem cells (hiPSCs) into the hippocampus of rats exhibiting chronic temporal lobe epilepsy (TLE) would: (1) greatly diminish the frequency and intensity of spontaneous recurrent seizures (SRS, Specific Aim 1); and (2) ameliorate learning and memory impairments and depressive-like behavior (Specific Aim 2). The work for this grant commenced from 02/04/2015 following the receipt of ACURO approval on 02/03/2015. During the past 8 months, a portion of experiments in Specific Aim 1 was performed: (1) Induction of status epilepticus (SE) in young rats through kainic acid injections to generate rats exhibiting chronic TLE typified by SRS. (2) Grafting of MGE-like precursors into the hippocampus of chronically epileptic rats. (3) Measurement of seizure-recordings through EEG recordings in chronically epileptic rats belonging to sham-grafting surgery, epilepsy-alone and grafted groups. The data collected so far suggested that animals in sham-grafting surgery and epilepsy-only groups have similar frequency and intensity of SRS, implying that sham-grafting surgery neither alleviates nor enhances the frequency and intensity of SRS. The effect of grafting on SRS will be quantified in the coming year once EEG data are collected from all experiments groups.

15. SUBJECT TERMS

Temporal lobe epilepsy, Human induced pluripotent stem cells, GABA-ergic precursor cells, Medial Ganglionic eminence, Memory dysfunction, Depression, Spontaneous Recurrent Seizures

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

	Page
1. Introduction……	4
2. Keywords……	4
3. Accomplishments…….……..	4
4. Impact……..……	11
5. Changes/Problems..……	11
6. Products…….……	11
7. Participants & Other Collaborating Organizations……	12
8. Special Reporting Requirements……	12
9. Appendices……	12

1. INTRODUCTION

Epilepsy, affects ~3 million Americans and ~60 million people globally. Military personnel are at a greater risk for developing temporal lobe epilepsy (TLE) because of increased incidences of traumatic brain injuries (TBIs). While antiepileptic drug (AED) therapy is effective for controlling seizures in many patients, ~35% of patients with TLE have chronic seizures that are resistant to AEDs. Besides, AED therapy has adverse side effects and does not reduce cognitive and mood impairments associated with TLE. Hence, alternative therapies having potential for both restraining spontaneous seizures and easing cognitive and mood impairments in chronic TLE are needed. Spontaneous recurrent seizures (SRS) in TLE originate from the temporal lobe foci and are associated with multiple epileptogenic changes in the hippocampus. Scarcity of inhibitory gamma-amino butyric acid (GABA)-ergic interneurons stands out among epileptogenic changes because higher levels of GABA are known to suppress the occurrences of seizures. Hence, the idea of restraining SRS in the epileptic hippocampus via grafting of cells that release the inhibitory neurotransmitter GABA has received much attention. The goal of this project is to examine whether grafting of medial ganglionic eminence (MGE)-like precursors generated from human induced pluripotent stem cells (hiPSCs) into the hippocampus of rats exhibiting chronic temporal lobe epilepsy (TLE) would: (1) greatly diminish the frequency and intensity of spontaneous recurrent seizures (SRS, Specific Aim 1); and (2) ameliorate learning and memory impairments and depressive-like behavior (Specific Aim 2).

2. KEYWORDS

Cognitive dysfunction
Cell therapy
Depression
Epilepsy
Spontaneous recurrent seizures (SRS)
GABA
GABA-ergic precursor cells
Hippocampus
Hippocampal neurogenesis
Human induced pluripotent stem cells
Medial ganglionic eminence (MGE)
Memory dysfunction
Stem cell differentiation
Stem cell proliferation
Stem cell therapy
Temporal lobe epilepsy

3. ACCOMPLISHMENTS

3.1. Major Goals: The major goals of this project are to examine whether grafting of MGE precursors generated from hiPSCs into the hippocampus of chronically epileptic rats would: (i) greatly diminish the frequency and intensity of SRS (Specific Aim 1 studies); and (ii) ameliorate learning and memory impairments and depressive-like behavior (Specific Aim 2 studies). ***Accomplishment of goals in Specific Aim 1*** would require the following experiments: (1) Raising rats exhibiting chronic TLE, typified by SRS: This is done through induction of status epilepticus (SE) via graded kainic acid injections, termination of acute seizures 2 hours after SE onset via diazepam injections and measurement of behavioral SRS

via direct observations at 3-4 months after SE. (2) Generation of MGE precursors from hiPSCs in culture: This is accomplished through a directed differentiation method, which is performed in the laboratory of our collaborator (Dr. Su-Chun Zhang (Univ of Wisconsin, Madison) and then shipped to our lab for grafting studies. (3) Preparation of cell suspension of human MGE precursor cells and transplantation into the hippocampus of rats exhibiting chronic TLE. This involves bilateral injections of MGE precursor cells into the hippocampus (3 sites/side) using stereotactic neurosurgery and daily cyclosporine injections thereafter until the collection of tissues through euthanasia. (4) Sham-grafting surgery: This involves bilateral injections of the culture medium into the hippocampus of rats exhibiting chronic TLE (3 sites/side). (5) Surgical implantation of epidural electrodes for electroencephalographic (EEG) recordings (survival surgery). (6) Continuous EEG recordings for three weeks from epileptic animals belonging to grafted, sham-surgery, cyclosporine alone and epilepsy-only groups. (7) Analyses of the survival, migration and differentiation of graft-derived cells and measurement of GABA and glutamate in the epileptic hippocampus belonging to different groups. ***Accomplishment of goals in Specific Aim 2*** would require the following experiments: experiments 1-4 described in Specific Aim 1 above plus the following experiments: (5) injections of 5'bromodeoxyuridine in the 4th week after grafting to label newly born cells and neurons in the hippocampus. (6) Measurement of functions such as learning, memory and mood using a series of behavioral tests. (7) Quantification of hippocampal neurogenesis and measurement of neurotrophic factors.

3.2. Studies Accomplished During the Past Year:

Duration of study: 02/04/2015 to 09/29/2015 (~8 months)

On February 3, 2015, we received a notification from the Department of the Army that the protocol PR130086 entitled, Human induced pluripotent stem cell (hiPSC)-derived GABA-ergic precursor cell therapy for chronic epilepsy", IACUC protocol number 2014-005, Protocol Principal Investigator Ashok Shetty, is approved by the USAMRMC Animal Care and Use Review Office (ACURO) as of 30-JAN-2015 for the use of rats and will remain so until its modification, expiration or cancellation. Upon receiving this notification, we commenced animal experiments for this study.

3.2.1. Major Activities: The following narrative describes studies accomplished so far for Specific Aim 1 (Task 1). The major goal of this aim is to determine whether grafting of MGE-like GABA-ergic precursors generated from hiPSCs into the hippocampus of chronically epileptic rats (Group 1) would diminish the frequency and intensity of SRS, in comparison to several control groups. The control groups comprise: sham-grafting surgery group (Group 2), cyclosporine only group (Group3), epilepsy-only group (Group 4).

3.2.2. Specific Activities:

(1) A total of 115 rats have been purchased so far in 3 different batches. We performed kainic acid (KA) injections to these rats to induce acute seizures or status epilepticus (SE) in 11 separate experimental sessions (n=8-12/session). These experiments belong to subtask 1a under Task 1 (Specific Aim 1)

(2) Of 115 rats, 102 rats developed the required SE (acute seizure activity for over two hours) and survived the procedure. Six (n=6) rats did not develop adequate SE (no stage IV or V seizures) and hence were excluded from the study. Five rats (n=5) died due to seizures during SE and one rat (n=1) was euthanized as it developed health issues and another rat (n=1) died during the required waiting period for the next step of experiments.

(3) Rats that survived SE procedure (n=102) were assigned to the next step, which is observation for the occurrences of behavioral spontaneous recurrent seizures (SRS). These experiments belong to subtask 1b under Task 1 (Specific Aim 1). Among 102 animals, scoring of behavioral SRS has been completed for 52 rats so far. The remaining 50 rats are now maintained in the vivarium. They will be scored for SRS in the coming quarter.

(4) From 52 animals that have completed behavioral SRS recordings, 11 rats were assigned to the sham-grafting surgery group, 12 rats to the epilepsy-only group, 25 rats to the grafting group and 4 rats to the cyclosporine alone group (Group 3 of Specific Aim 1).

(5) Experiments pertaining to the Sham-Surgery Group (Group 2 of Specific Aim 1): We performed sham-grafting survival surgery to animals in this group (n=11) over 2-3 surgery sessions (Subtask 1c of Task 1). This involved bilateral injections of the cell culture medium into the hippocampus (3 sites on each side) using appropriate stereotaxic coordinates. We also implanted electrodes for electroencephalographic (EEG) recordings in these rats in the same surgery sessions. In the subsequent post-surgery survival period, one rat died and 3 rats lost their implanted EEG electrodes (due to seizure-related accidents in the cage while housing), which left 7 rats for chronic EEG recordings in this group. All 7 rats were connected to the EEG machine and chronic EEG recordings have been taken continuously for 3 weeks (Subtask 1d of Task 1). Data for 6 rats from 8 days of EEG recordings have been analyzed so far, which are presented in this report. The remaining data from stored EEG recordings will be analyzed in the coming quarters.

(6) Experiments pertaining to the Epilepsy-Only Group (Group 4 of Specific Aim 1): We performed EEG electrode implantation surgery to animals assigned to this group (n=12) over 2-3 surgery sessions (Subtask 1c of Task 1). In the subsequent post-surgery survival period, one rat died and 4 rats lost their implanted EEG electrodes (due to seizure-related accidents in the cage while housing), which left 7 rats for chronic EEG recordings in this group. All 7 rats were connected to the EEG machine and chronic EEG recordings have been obtained for 3 weeks (Subtask 1d of Task 1). Data for 6 rats from 30 hours of EEG recordings have been analyzed so far, which are presented in this report. The remaining data from stored EEG recordings will be analyzed in the coming quarters.

(7) Experiments pertaining to the Graft Group (Group 1 of Specific Aim 1): The experiment for this group involves grafting of GABA-ergic precursor cells expanded from human induced pluripotent stem cells (hiPSCs) (Subtask 1c of Task 1): So far, we performed grafting to 17 chronically epileptic rats in 5 surgery sessions. Out of these, 2 rats died and 4 rats lost their implanted electrodes during the waiting period for the next step of experiments. Another rat was found to be sick and hence excluded from the study. We performed EEG recordings from 2 grafted rats until now (Subtask 1c of Task 1). Data from these rats will be analyzed in the coming quarter. The remaining grafted animals (n=8) now waiting for EEG recordings. This will be accomplished in the next quarter.

Thus, we have completed ~40% of experiments pertaining to Specific Aim 1 so far (i.e. during the 8-month of funding period).

3.2.3. Progress Details: We focused on experiments belonging to Specific Aim 1 (Task 1). We performed experiments described in "Subtask 1a", "Subtask 1b", "Subtask 1c" and "Subtask 1d".

3.2.3.1. Experiments performed for Subtask 1a: A total of 115 rats were purchased in 3 different batches. We performed KA injections to induce acute seizure activity or SE (as per the approved protocol) in 11 separate experimental sessions (n=8-12/session). Of these rats, 102 developed the required SE (acute seizure activity for >2 hours) and survived the procedure.

Six (n=6) rats did not develop adequate SE (i.e. lack of stages IV or V seizures) and hence were excluded from the study. Five rats (n=5) died due to seizures during SE. One rat (n=1) was euthanized as it developed health issues and another rat (n=1) died during the required waiting period for the next stage of experiments.

Brief Methodology: This procedure involves 3-5 low-dose graded intraperitoneal injections of KA (3.0mg/Kg/hour for 3-5 hours). This results in continuous stages III-V seizures (i.e. SE) in ~90% of rats. These seizures are typified by unilateral forelimb clonus (Stage III), bilateral forelimb clonus (Stage IV) and bilateral forelimb clonus with rearing and falling (Stage V). After 2 hours of continuous stages III-V seizure activity, an intraperitoneal injection of diazepam (5 mg/Kg) is administered to terminate behavioral seizures, which also reduces the mortality associated with SE.

3.2.3.2. Experiments performed for Subtask 1b: Rats that survived SE procedure (n=102) were assigned to the next step, which is observation for the occurrences of behavioral spontaneous recurrent seizures (SRS). Among 102 animals, scoring of behavioral SRS has been completed for 52 rats (23 rats from the first batch and 29 rats from the second batch) in the 3rd month after SE through direct observations for 48 hours over 3-4 week time-period. All of these rats (n=52) displayed SRS. The various parameters of behavioral spontaneous seizures such as the frequency of all SRS, the frequency of stage V-SRS (the most severe type of seizures), and the average duration of individual seizures were calculated for all animals before using for further experiments.

Behavioral SRS Scores

(i) Frequency of all SRS	0.27 ± 0.03/hour (~6.5 seizures/day)
(ii) Frequency of Stage V-SRS	0.21 ± 0.03/hour (~5.0 seizures/day)
(iii) Duration of individual SRS	34.91 ± 0.92 seconds

From these 52 animals that have completed behavioral SRS recordings, 11 rats were assigned to the sham-grafting surgery group (Group 2), 12 rats to the epilepsy-only group (Group 4), 25 rats to the grafting group and 4 rats to the cyclosporine alone group (Group 3).

The remaining 50 rats are now maintained in the vivarium. They will be scored for SRS in the coming quarter. Rats that display significant SRS will be used to complete the remaining experiments for Specific Aim 1 (Task 1). Once the total number of epileptic rats required for Specific Aim 1 studies are met, the remaining rats will be employed for Specific Aim 2 studies during the coming year (task 2).

Brief Methodology: This involves direct observations of animals for behavioral seizures in 4 or 6-hour sessions over 3-4 weeks for 48 hours. This is done to confirm robust chronic epilepsy development after SE.

3.2.3.3. Experiments performed for Subtask 1c:

(a) Sham-Surgery Group (Group 2 of Specific Aim 1): We performed sham-grafting survival surgery to animals assigned to this group (n=11) over 2-3 surgery sessions. This involved bilateral injections of the cell culture medium into the hippocampus (3 sites on each side) using appropriate stereotaxic coordinates. We also implanted electrodes for EEG recordings in these rats. In the subsequent post-surgery survival period, one rat died and 3 rats lost their implanted EEG electrodes leaving 7 rats in this group for chronic EEG recordings.

Brief Methodology*:* Sham surgery involves aseptic survival surgery using a stereotactic apparatus, as detailed in the approved animal protocol. Each animal is deeply anesthetized and fixed to a stereotactic device. Then burrholes are made in the skull using appropriate coordinates to inject the culture medium into the hippocampus on both sides. Post-operative care follows in the subsequent days after surgery. EEG electrode fixing surgery involves drilling

of additional burrholes in the skull to implant metal EEG recording electrodes with mounting screws to the epidural space, attachment of electrodes to the electrode pedestal, and fixing the electrode pedestal to the skull using dental cement and post-operative care in the subsequent days.

(b) Epilepsy-Only Group (Group 4 of Specific Aim 1): We performed EEG electrode implantation surgery to animals assigned to this group (n=12) over 2-3 surgery sessions. In the subsequent post-surgery survival period, one rat died and 4 rats lost their implanted EEG electrodes leaving 7 rats in this group for EEG recordings.

Brief Methodology: EEG electrode fixing surgery involves aseptic survival surgery using a stereotactic apparatus, as detailed in the approved animal protocol. Each animal is deeply anesthetized and fixed to a stereotactic device. Then burrholes are made in the skull using appropriate coordinates to implant metal EEG recording electrodes with mounting screws to the epidural space, attachment of electrodes to the electrode pedestal, and fixing the electrode pedestal to the skull using dental cement and post-operative care in the subsequent days.

(c) Grafted Group (Group 1 of Specific Aim 1): We grafted GABA-ergic precursor cells derived from the human induced pluripotent stem cells (hiPSCs) into 17 rats so far. These rats were chosen from a group of 52 rats that underwent SE and displayed robust SRS in the 3rd month after SE (described above under, "Experiments performed for Subtask 1b"). These rats displayed similar SRS (~0.25 all SRS/hour) as rats in sham-surgery and epilepsy-only groups. The donor cells for grafting were generated from hiPSCs through directed differentiation methods. These cells were provided by our collaborator (Dr. Su-Chun Zhang, University of Wisconsin, Madison, WI), which is described in the grant proposal as well as Statement of Work. Out of total 17 rats grafted with hiPSC derived GABA-ergic precursor cells, 2 rats died and 4 rats lost their implanted electrodes. One rat was found to be sick and hence was excluded from the study. So far, EEG recordings have been completed for 2 grafted rats. Data will be analyzed in the next quarter. In addition, EEG recordings from all other grafted rats will be performed in the next few coming quarters.

Brief Methodology: Grafting surgery involves aseptic survival surgery using a stereotactic apparatus, as detailed in the approved animal protocol. Each animal is deeply anesthetized and fixed to a stereotactic device. Then burrholes (3 per side) are made in the skull using appropriate coordinates to inject GABA-ergic precursor cells into the hippocampus on both sides. Post-operative care follows in the subsequent days after surgery. All grafted rats are currently receiving daily subcutaneous injections of an immunosuppressant drug cyclosporine at 10mg/Kg, since xeno-grafting (grafting of human cells into the rat brain) is performed in these studies.

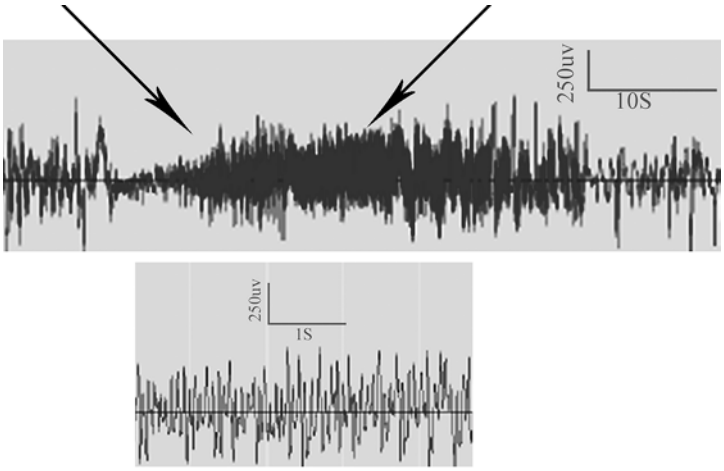
3.2.3.4. Experiments performed for Subtask 1d:

(a) Sham-Surgery Group: All rats that retained their implanted EEG electrodes in this group (n=7) were connected to the EEG machine and EEG recordings were obtained continuously for 3 weeks. Data for 6 rats from 8 days of EEG recordings have been analyzed so far, which are presented below. The remaining data from stored EEG recordings will be analyzed in the coming quarters.

EEG-SRS Scores for Sham-Surgery Group (n=6):

(i) Frequency of all EEG-SRS	0.72 ± 0.05/hour (~17 seizures/day)
(ii) Frequency of Stage V-SRS	0.67 ± 0.04/hour (~16 seizures/day)
(iii) Duration of individual SRS	44.3 ± 2.8 seconds
(iv) Total time spent in seizures	0.9 ± 0.07% of recorded time

EEG Trace during a spontaneous recurrent seizure (SRS) in an animal that underwent sham-grafting surgery (in the chronic phase of epilepsy):



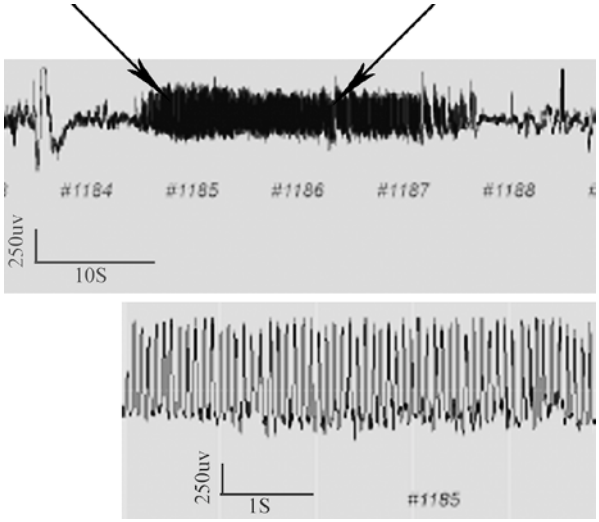
High frequency, High-voltage, Synchronized Polyspikes

(b) Epilepsy-Only Group: All rats that retained their implanted EEG electrodes in this group (n=7) were connected to the EEG machine and EEG recordings were obtained continuously for 3 weeks. Data for 6 rats from 30 hours of EEG recordings have been analyzed so far, which are presented below. The remaining data from stored EEG recordings will be analyzed in the coming quarters.

EEG- SRS Scores:

(i) Frequency of all SRS	0.55 ± 0.09/hour (~13 seizures/day)
(ii) Frequency of Stage V-SRS	0.53 ± 0.1/hour (~12.7 seizures/day)
(iii) Duration of individual SRS	43.5 ± 7.23 seconds
(iv) Total time spent in seizures	0.7 ± 0.12% of recorded time

EEG Trace during a spontaneous recurrent seizure (SRS) in an animal that was maintained as epilepsy-only rat (in the chronic phase of epilepsy):



High frequency, High-voltage, Synchronized Polyspikes

Thus, from the EEG data collected so far, animals in sham-grafting surgery group and epilepsy-only group seem to have similar frequency and intensity of SRS. These results imply that sham-grafting surgery in the chronic phase of epilepsy neither alleviates nor

enhances the frequency and intensity of SRS. However, a final conclusion on this would need analyses and inclusion of data from all three weeks of recordings. This will be accomplished in the next quarter.

(c) Grafted group: Among 17 rats grafted with hiPSC derived GABA-ergic precursor cells, 2 rats died, 4 rats were lost their implanted electrodes and one rat was excluded from the study because of sickness. So far, EEG recordings have been obtained from 2 rats. The EEG data will be analyzed in the coming quarter. In addition, EEG recordings from remaining grafted rats will be accomplished in the coming quarters.

Brief Methodology employed in Subtask 1d: Each rat is placed in a Plexiglas cage, the connector cable of the video EEG system is fixed to the electrode pedestal on the rat's head. The video-EEG system will continuously monitor simultaneously occurring behavior and electrographic activity in freely behaving animals. As the connector cable of the EEG system passes through a swivel, animals can move, eat or drink without any restraint within the cage.

3.3. Opportunities for Training and Professional Development:

Newly hired full time Senior Research Associate (Dr. Dinesh Upadhya) for this project has received considerable training for the various specialized experimental approaches and techniques from the PI (A. K. Shetty, Professor) and/or a senior researcher (Dr. B. Hattiangady, Assistant Professor) working for this project. These include induction of SE, measurement of behavioral SRS, Culturing and characterization of human iPSCs and MGE precursors derived from them, transplantation neurosurgery, implantation of EEG electrodes and chronic EEG recordings using a tethered EEG system, EEG data collection and analyses. Professional development activity for key research personnel in this project mainly comprised participation in departmental seminars and journal clubs, and discussion on epilepsy and stem cell therapy research advances with the PI on a regular basis. In addition, both B. Hattiangady and Dr. Upadhya will be attending the Society for Neuroscience conference at Chicago this year.

3.4. Dissemination of Results to Communities of Interest:

Nothing to Report

3.5. Plans for the Next Reporting Period:

In the coming year, we will continue and complete Task 1 (Specific Aim 1) experiments detailed in Subtasks 1b-1d and 1e-1f (immunohistochemical and biochemical studies). Specifically, we will collect the required EEG seizure data from animals belonging to all groups in Specific Aim 1 so that the final seizure values can be compared statistically between groups. This would ascertain whether human MGE precursor cell grafting to the hippocampus of chronically epileptic rats would considerably ease SRS. Additionally, these experiments would provide information on the survival and differentiation of human precursor cells in the chronically epileptic rat hippocampus.

Subtask 1b experiments*:* We will assess behavioral spontaneous recurrent seizures in animals that survived status epilepticus from the third batch (n=50). We will select animals that display SRS (i.e. chronic epilepsy) for further experimentation. We will first assign the required number of rats to different groups in Specific Aim 1.

Subtask 1c experiments: Rats for the cyclosporine only group will be implanted with EEG electrodes and will receive daily cyclosporine injections.

Subtask 1d experiments: We will perform EEG recordings from animals that were grafted with hiPSC derived MGE-like GABA-ergic progenitor cells as well as from epileptic rats receiving cyclosporine alone.

Subtask 1e and 1f experiments: Brain tissues will be harvested from epileptic animals belonging to the grafted, sham-surgery, cyclosporine-alone and epilepsy-only groups, following completion of EEG recordings. These tissues will be used to analyze the survival and differentiation of graft-derived cells (using multiple immunocytochemical methods) and/or analyses of glutamate and GABA in host tissues.

Once the above experiments are completed, we will commence animal experiments for Specific Aim 2 in the later part of year 2.

4. IMPACT:

Nothing to Report

5. CHANGES AND PROBLEMS:

(i) Changes in approach:

No changes in approach were required during the past year. None anticipated for the coming year.

(ii) Actual or Anticipated Problems or Delays and Plans to Resolve them:

As per the notice of grant award, this project commenced from 09/30/2014. Nonetheless, since the ACURO approval was received on 02/03/2015, the research work for this project actually commenced from 02/04/2015. Thus, the experiments for this project commenced from the 2nd month of 2nd quarter in the first year. Hence, ~8 months of work has been performed for this project during the past year. It is anticipated that additional time (beyond the scheduled 3 years) will be required to complete all studies proposed in this project.

(iii) Changes that had a significant impact on expenditures:

Nothing to Report

(iv) Significant Changes in the use of vertebrate animals or biohazards:

Nothing to Report

(v) Significant Changes in the Care of Vertebrate Animals:

Nothing to Report

6. PRODUCTS:

Publications:

(1) A review article on GABA-ergic cell therapy has been submitted for publication in a peer-reviewed journal, which is currently in revision after an initial peer-review.

Shetty, A.K. GABA-ergic Cell Therapy for Epilepsy: Advances, Limitations and Challenges. ***Neuroscience and Biobehavioral Reviews*** (currently in revision after peer review), 2015. Federal Support is acknowledged.

7. PARTICIPANTS AND OTHER COLLABORATIVE ORGANIZATIONS

The following research staff members from PI's laboratory were compensated from this grant (for the percentage of effort contributed to this project)

Personnel	Role	Percent Effort
Ashok K. Shetty	Principal Investigator	17%
Bharathi Hattiangady	Research Scientist	48%
Dinesh Upadhya	Senior Research Associate	90%
Xiaolan Rao	Research Assistant	50%

Other Collaborators:

Su-Chun Zhang Lab (University of Wisconsin, Madison)

Dr. Zhang's laboratory has provided the required donor cells (MGE-like cells derived from hiPSCs) for grafting studies in this project, as approved in the project. A sub award to the University of Wisconsin, Madison has been approved and implemented at the commencement of this project.

Changes in active other support of the PI or Key Personnel:

Nothing to Report

Other Organizations Involved in this Project:

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART is attached (Page 13 of this document)

9. APPENDICES

QUAD CHART is attached (Page 13 of this document)

Title: **Human iPSC-Derived GABA-ergic Precursor Cell Therapy for Chronic Epilepsy**
ERMS/Log Number and Task Title: CDMRP Log Number, **PR130086**
Insert Award Number: Grants.gov ID Number, **GRANT11498566**

PI: Ashok K. Shetty, PhD **Org:** Texas A&M University System Health Science Center **Award Amount:** $908,423

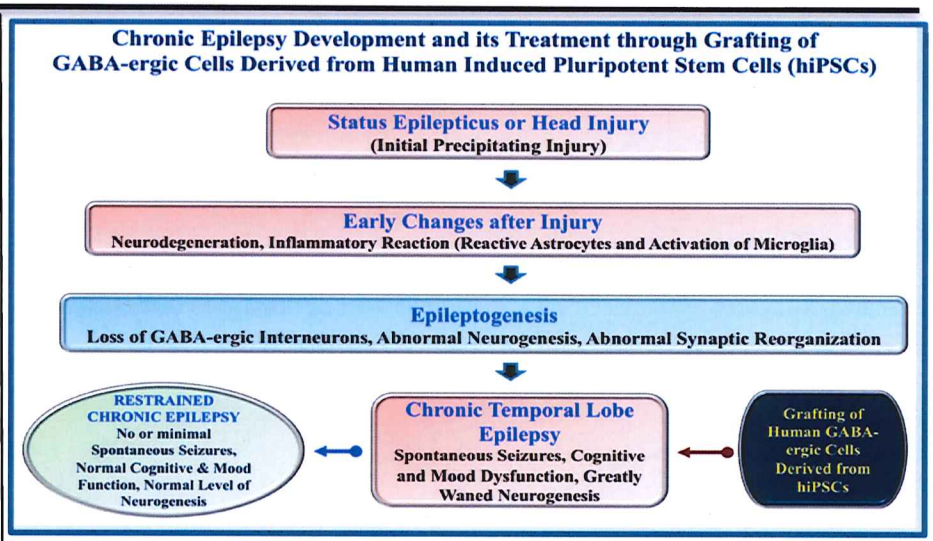
Study Aims

Specific Aim 1: Test the hypothesis, "Grafting of medial ganglionic eminence (MGE)-like gamma amino butyric acid (GABA)-ergic precursors from human induced pluripotent stem cells (hiPSCs) into the hippocampus of chronically epileptic rats (CERs) greatly diminishes the frequency and intensity of spontaneous recurrent seizures (SRS).

Specific Aim 2: Address the hypothesis, "Grafting of MGE-like GABA-ergic precursors from hiPSCs into the hippocampus of CERs greatly improves learning and memory function and reverses depressive-like behavior.

Approach

We will generate MGE precursors from hiPSCs in culture. We will then prepare a suspension of these cells and transplant into the hippocampus of rats exhibiting chronic epilepsy. In studies pertaining to Specific Aim 1, we will quantify the effects of grafts on the frequency, severity and duration of SRS via chronic video-electroencephalographic (video-EEG) recordings. In Specific Aim 2, we will quantify the effects of grafting on functions such as learning, memory and mood.



Accomplishment: ~40% or work pertaining to Specific Aim 1 Studies.

Timeline and Cost

Activities CY	14	15	16	17
Aim 1: Induction of seizures, Grafting of GABA-ergic cells, EEG recordings				
Aim 1: Grafting of GABA-ergic cells, EEG Recordings, Histology, Immunohistochemistry				
Aim 2: Induction of seizures, Grafting of GABA-ergic cells, Behavioral tests, Analyses of neurogenesis				
Estimated Budget, total cost ($K)	**$304K**	**$300K**	**$304K**	

Updated: (Temple, TX, October 13, 2015)

Goals/Milestones

CY14 Goals - Generate rats with chronic epilepsy
- ☑ Induction of acute seizures

CY15 Goals – Grafting cells into epileptic rats; testing its effect on seizures
- ☑ Induction of acute seizures; Monitoring of behavioral seizures
- ☑ Intracerebral grafting of human GABA-ergic cells
- ☑ EEG Recordings
- ☐ Histology; Immunohistochemistry (analyses of grafts)

CY16 Goals – Grafting cells into epileptic rats; testing its effect on seizures
- ☐ Intracerebral grafting of human GABA-ergic cells
- ☐ EEG Recordings
- ☐ Histology; Immunohistochemistry (analyses of grafts)
- ☐ Induction of acute seizures; Monitoring of behavioral seizures for Aim 2 studies

CY17 Goals – Grafting cells into epileptic rats; testing its effect on behavior
- ☐ Intracerebral grafting of human GABA-ergic cells
- ☐ Behavioral tests for Learning, Memory and Mood function
- ☐ Analyses of Hippocampal Neurogenesis

Budget Expenditure to Date: **$178,301.07 + 54,944 (sub-award), $233,245.07 total**